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Serum Amyloid A Protein in the Prediction of Postburn Complications and Fatal Outcome in Patients with Severe Burns

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Summary: Fifty adults hospitalized with extensive burns formed the basis of the present study. Serum amyloid A protein, C-reactive protein, α_1-antichymotrypsin and α_1-acid glycoprotein were measured in serum samples taken on admission, and 3 and 7 days later. Fatal outcome was observed in 13 out of 14 (93%) patients with serum amyloid A protein over 100 mg/l on admission and in only 2 of the remaining 36 (6%) patients with serum amyloid A protein below 100 mg/l. The median serum amyloid A protein concentration on admission in 15 patients with fatal outcome was 134 mg/l, and only 30 mg/l in 35 patients who recovered ($p < 0.00005$). As a reference value, the level of 100 mg serum amyloid A protein per litre on admission gave an evident predictive value (93%) and sensitivity (87%) for fatal outcome. The difference between serum amyloid A protein concentrations in patients with complications (median 642 mg/l) and those without complications (median 250 mg/l) was statistically very significant ($p = 0.0003$) three days after burn injury. The level of 400 mg/l as a reference value 3 days after burn injury gave a reasonable predictive value (80%) and sensitivity (74%) for the development of postburn complications, but patients who died did not develop a hypermetabolic reaction and their serum amyloid A protein concentration remained below 400 mg/l, despite high serum amyloid A protein concentrations observed on admission (above 100 mg/l). No statistical significance was observed for the other 3 acute phase proteins investigated in this study.

Introduction

Thermal injury is caused by overheating of body tissues above the critical temperature, leading to tissue damage. The pathophysiology of the burn syndrome is characterized by the burn wound, its infection and the host's impaired defence to it, hypermetabolism and increased energy demand. Vital postburn complications are: pulmonary insufficiency, renal insufficiency and complications involving the gastrointestinal tract (1).

Tissue damage, including burns, leads to considerable changes in plasma proteins, a reaction commonly referred to as "the acute phase response". The concentration of many plasma proteins, derived largely from the liver, changes during inflammation. These are known as acute phase proteins. Severely burned patients display a strong acute phase response in the first hours to days after burn injury. They often have a high fever within 24 hours and produce acute phase proteins (2). A reliable marker of acute phase response must fulfill the following criteria: a rapid and substantial increase in concentration following an inflammatory stimulus and a short half-life in circulation (3). Although some 34 known plasma proteins increase in concentration during inflammation, only two of them are suitable markers of acute phase response: serum amyloid A protein and C-reactive protein (4–6). Both are quick to respond and both increase substantially in severe inflammation, but serum amyloid A protein is the more sensitive quantity (6–9). It shows a more than 1000-fold increase in plasma concentration a few hours (4–6) after inflammatory stimulus (3), and has a very short half life of between 75 and 90 minutes (10, 11). Serum amyloid A protein is an apolipoprotein of very low density (VLDL) and high density lipoproteins (HDL) (12) and may have an important role in the removal of excess cholesterol from phagocytosing macrophages. It is probably a recognition marker for liver uptake of HDL-bound cholesterol, hence its clearance from the circulation (3). It has been shown that serum amyloid A protein-enriched HDL are catabolized more rapidly than normal HDL. Possibly HDL and perhaps also VLDL may function to remove non-polar toxins or cellular constituents from sites of injury and transport them to the liver for detoxification (13).

The aim of the present study was to evaluate the behaviour of serum amyloid A protein together with the other three most important acute phase proteins: C-reactive protein, α_1-antichymotrypsin and α_1-acid glycoprotein in the early phases of severe burns, in order to determine the statistical significance of these biochemical indicators for the early prediction of postburn complications and fatal outcome.

Patients and Methods

The study group comprised 50 adults (aged between 21 and 82 years, mean age 45) with total body surface area burns greater than 20% (range from 20 to 95%, mean 42%). All patients received a uniform regime of treatment consisting of standard fluid resuscitation, nutritional support, plasma expanders, fresh frozen plasma and prophylactic antibiotic therapy. A topical antimicrobial agent, silver sulfadiazine, was applied to delay the onset of and decrease the level of bacterial colonization. The first blood samples were taken immediately upon admission and subsequently on days 3 and 7 after the burn injury. Sera from patients who developed complications were taken every day during that period. Sera were stored at −20 °C and assayed in batches at the end of the study. Serum amyloid A protein concentrations were determined by the micro ELISA test (14) with a sensitivity of 0.1 mg/l and precision expressed as coefficients of variation between 1.6 and 4.2%. Immunoaffinity-purified sequence-specific antibody against serum amyloid A protein (15) was obtained from Tinolab (Zagreb, Coratia). C-reactive protein, α_1-antichymotrypsin and α_1-acid glycoprotein were determined by the method of *Mancini* et al. (16) with a sensitivity of 5 mg/l and coefficients of variation below 5%. Specific antibodies against C-reactive protein were obtained from Roche Diagnostica (Basel, Switzerland) while specific antibodies against α_1-antichymotrypsin and α_1-acid glycoprotein, human serum C-reactive protein calibrator and human serum protein calibrator (for α_1-antichymotrypsin and α_1-acid glycoprotein standardization) were purchased from Dako (Glostrup, Denmark). The statistical significance was calculated using the *Mann-Whitney* U test.

Results

Figure 1 shows the changes of the median concentrations of all four acute phase proteins during the course of 7 days. Altogether 27 patients (54%) developed postburn complications and 15 patients (30%) had fatal outcome. The average age of the survivors was 40 years, and that of the non-survivors was 55 years. The survivors had less severe burns, with a mean total body surface area burn of 37%, while the non-survivors had a mean total body surface area burn of 46%.

According to these findings, patients were divided into two groups: survivors (35 patients) and non-survivors (15 patients). The same patients were again divided into two further groups: those who developed postburn complications (27 patients) and those who did not (23 patients). Results are presented in figure 2.

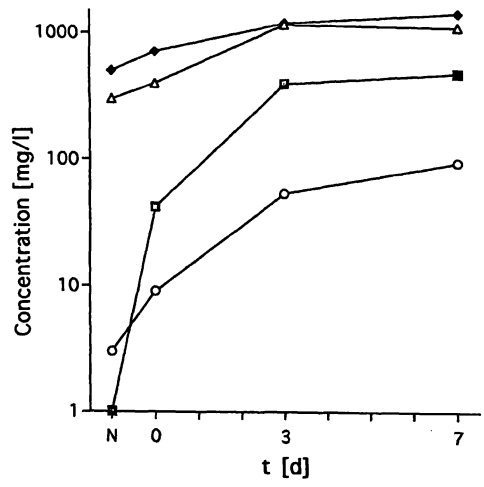

Fig. 1 Seven days follow-up of: serum amyloid A protein (■), C-reactive protein (○), α_1-antichymotrypsin (△) and α_1-acid glycoprotein (◆) concentrations in 50 patients with severe burns. Each data point is the group median value. N denotes normal value.

Fatal outcome was observed in 13 out of 14 (93%) patients with serum amyloid A protein concentrations above 100 mg/l on admission and in only 2 of the remaining 36 (6%) patients with serum amyloid A protein concentrations below 100 mg/l. The median serum amyloid A protein concentration on admission in 15 patients with fatal outcome was 134 mg/l and only 30 mg/l in 35 patients that recovered ($p < 0.00005$, $U = 70$); the normal mean (reference) value for 30 healthy adults was 0.8 mg/l, range < 0.1−1.2 mg/l. The difference between serum amyloid A protein concentrations in patients who later developed complications (with median serum amyloid A protein value of 642 mg/l on day 3 and 1050 mg/l on day 7) and those who did not (with median serum amyloid A protein value of 250 mg/l on day 3 and 175 mg/l on day 7) was statistically very significant ($p = 0.0003$, $U = 125$ and $p < 0.00005$, $U = 85$, three and seven days after burn injury, respectively).

Fatal outcome was observed in 9 out of 23 (39%) patients with C-reactive protein values above 10 mg/l on admission and in 6 out of the remaining 27 (22%) patients with C-reactive protein values below 10 mg/l. The median C-reactive protein concentration on admission in 15 patients with fatal outcome was 17 mg/l and 9 mg/l in 35 patients that recovered ($p = 0.221$, $U = 205$); mean value of 30 healthy adults was below 5 mg/l. There was no statistically significant difference on admission or three days later between C-reactive protein values in patients who developed complications and those who did not (median C-reactive protein values of 60 and 53 mg/l on day 3, respectively), but it was very high 7 days after burn injury ($p < 0.00005$, $U = 82$; with median C-reactive protein values of 134 and 40 mg/l, respectively).

Fatal outcome was observed in 9 out of 21 (43%) patients with α_1-antichymotrypsin values above 0.40 g/l on admission and in 6 out of remaining 29 (21%) patients with α_1-antichymotrypsin values below 0.40 g/l. The median α_1-antichymotrypsin value of admission in 15 patients with fatal outcome was 0.47 g/l and 0.39 g/l in 35 patients that recovered ($p = 0.015$, $U = 180$). There was no statistically significant difference on admission between α_1-antichymotrypsin values in patients who developed complications and those who did not, but significant differences were observed 3 and 7 days after burn injury ($p = 0.0452$, $U = 203$ and $p = 0.017$, $U = 181$, respectively).

Fatal outcome was observed in 10 out of 25 (40%) patients with α_1-acid glycoprotein concentrations above 0.70 g/l on admission and in 5 out of the remaining 25 (20%) patients with α_1-acid glycoprotein values below

0.70 g/l. The median α_1-acid glycoprotein value on admission in 15 patients with fatal outcome was 0.76 g/l and 0.70 g/l in 35 patients that recovered, with no statistically significant difference. There was also no statistically significant difference during the investigated period between patients who developed complications and those who did not.

There was no correlation among the concentrations of acute phase proteins on admission or later.

Discussion

Our results show that severe burns induce a dramatic acute phase response within the first hours of injury. Peak levels of serum amyloid A protein and C-reactive protein were increased up to 3000- and 100-fold above normal concentrations, respectively. α_1-Antichymotrypsin and α_1-acid glycoprotein peak levels were increased up to sixfold above their normal values. These findings are very similar to our previous observations with acute phase proteins in patients with acute myocardial infarction (8). On admission to hospital, the ratio between serum amyloid A protein and C-reactive protein levels was usually between 5 : 1 and 11 : 1, which is similar to those observed during kidney allograft rejection (10 : 1) (17–19), influenza (11 : 1) or pneumonia (7 : 1) (9). The ratio between serum amyloid A protein and C-reactive protein was more increased 7 days after burn injury, and in same cases with postburn complications it was higher than 30 : 1, which we believe is the highest ratio ever reported, although we measured higher individual serum amyloid A protein concentrations in the sera of patients with acute myocardial infarction (9). In patients who developed postburn infections or sepsis, we found higher serum amyloid A protein values and a higher serum amyloid A protein/C-reactive protein ratio than we had observed earlier in patients with urinary tract infections (5 : 1) (6) or in neutropenic patients with acute leukaemia during their infectious febrile episodes (1 : 1 to 5 : 1) (7).

Results from this study suggest that postburn complications and fatal outcome are closey correlated with the serum amyloid A protein values determined on admission and three days later. As a reference value, the level of 100 mg/l on admission gave an evident predictive value (93%) and sensitivity (87%) for fatal outcome. The level of 400 mg/l, as a reference value 3 days after burn injury, gave a reasonable predictive value (80%) and sensitivity (74%) for the development of postburn complications. Patients who died did not develop a hypermetabolic reaction and their serum amyloid A protein levels remained below 400 mg/l, despite high levels (above 100 mg/l) on admission.

It was not possible to identify any C-reactive protein level on admission or later which would give a reasonable predictive value and sensitivity for mortality rate. The best results on admission were obtained with a level of 10 mg/l but with a poor predictive value (39%) and sensitivity (60%). Seven days later a level of 100 mg/l as reference value gave a good sensitivity for mortality

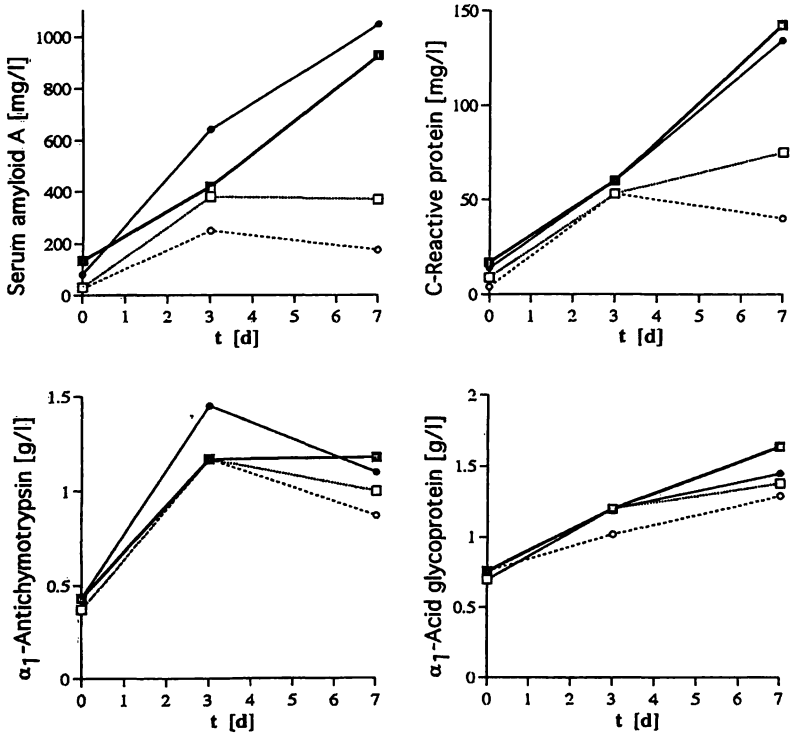

Fig. 2 Comparison among acute phase protein concentrations in four groups of patients with severe burns: patients with fatal outcome (■) and survivors (□); patients who developed postburn complications (●) and patients who recovered without complications (○). Each data point is the group median value.

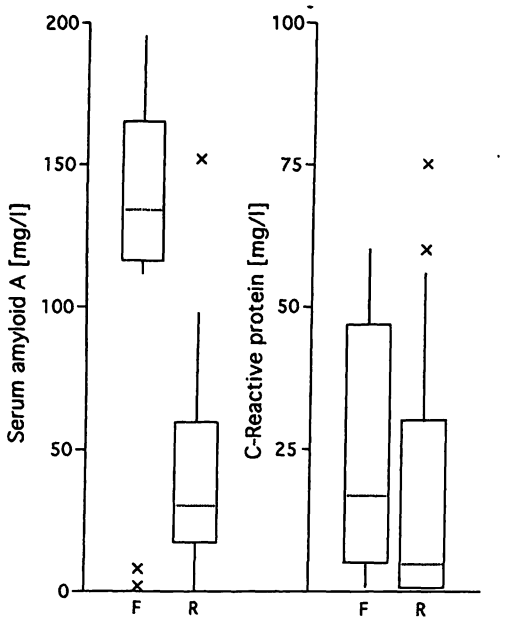

Fig. 3 Multiple box-and-whisker plot for serum amyloid A protein and C-reactive protein values on admission. Group F = patients with fatal outcome; group R = patients who recovered (survivors).

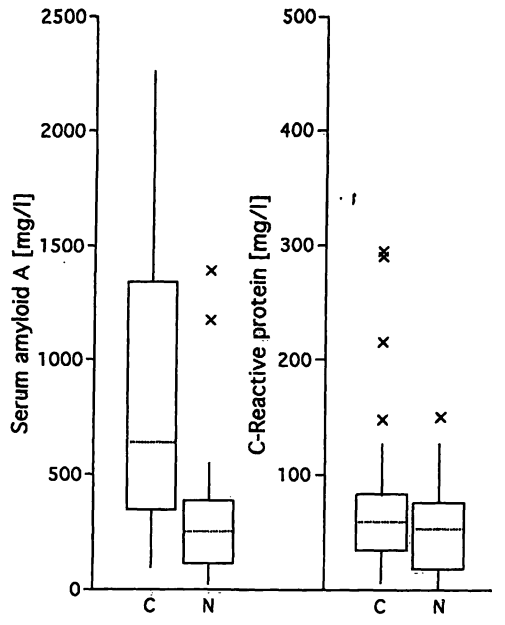

Fig. 4 Multiple box-and-whisker plot for serum amyloid A protein and C-reactive protein values three days after admission. Group C = patients who developed postburn complications; group N = patients who did not develop postburn complications.

rate (11/15; 73%) with no predictive value (11/24; 46%) but with a good predictive value (20/24; 83%) and sensitivity (20/27; 74%) for developing postburn complications.

Similarly, it was not possible to identify an α_1-antichymotrypsin level on admission or later which would give a reasonable predictive value and sensitivity for mortality rate. The best results on admission were obtained with a level of 0.40 g/l but with a poor predictive value (43%) and sensitivity (60%). As a reference value, 1.00 g/l gave a good sensitivity for mortality rate (12/15; 80%) with no predictive value (12/27; 44%) but with a poor predictive value and sensitivity (18/27; 67%) for developing postburn complications.

There was no α_1-acid glycoprotein level on admission or later which would give a reasonable predictive value and sensitivity for mortality rate. The best results were obtained with a level of 0.70 g/l on admission but with a poor sensitivity (67%) and no predictive value (40%).

According to the results presented in this study, we conclude that serum amyloid A protein is the best of all the four acute phase proteins investigated as a marker for early prediction of postburn complications and fatal outcome, even in the very early phase. Negligible overlap of serum amyloid A protein data in multiple box-and-whisker plots presented in figures 3 and 4 suggests that serum amyloid A protein is a very useful clinical laboratory quantity for differentiating patients with severe burns at the very beginning of the injury.

Acknowledgements

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